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In vitro *Actinomyces israelii* biofilm development on IUD copper surfaces

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Abstract

Background: Female pelvic actinomycosis may involve fallopian tubes, ovaries, uterus and bladder. This condition is often associated with the use of intrauterine contraceptive devices (IUDs), vaginal pessaries and/or tampons. The predominant causative agent of human actinomycosis is *Actinomyces israelii*, which has been found on copper IUDs retrieved from patients.

Study Design: In this work, a biofilm of *A. israelii* was developed in vitro on copper surfaces immersed in a simulated uterine fluid under anaerobic conditions. The biofilm was characterized using scanning electron microscopy (SEM), energy dispersive X-ray and atomic force microscopy.

Results: The capacity of *A. israelii* to develop a biofilm over copper surfaces in synthetic media was demonstrated. SEM micrographies illustrate the exopolysaccharides production and bacterial distribution.

Conclusion: *A. israelii* was able to attach and grow in synthetic intrauterine media and to present on the copper surface is likely due to the production of biofilm.

Keywords: Actinomycosis; *Actinomyces israelii*; Biofilm; Intrauterine device

1. Introduction

Actinomyces species are Gram-positive anaerobic bacteria that are normal inhabitants of the mouth, bowel level and lower reproductive tract. There are at least six species that can cause human disease of which *Actinomyces israelii* is the most common. Because *Actinomyces* are present as commensal organisms in healthy humans, they are best thought of as opportunistic pathogens [1]. The most common infection sites for clinical actinomycosis include cervicofacial (60%), thoracic (15%) and abdominal/pelvic (25%). Female genital actinomycosis is associated with the IUD and vaginal pessary. Roughly 7% of women with an IUD have *Actinomyces spp.* on their Pap smear examination [2]. Prolonged presence of an IUD is associated with pelvic presence of *Actinomyces.*

Actinomyces species grow as colonies that may extend slowly across natural anatomic boundaries, forming abscesses and sinus tracts filled with polymorphonuclear leukocytes and calcium phosphate [3]. The rate of colonization with *Actinomyces* in cytologic smears of IUD users noted in literature is wide, ranging from 1.6% to 44% [4].

Pelvic actinomycosis most commonly occurs in current or previous users of an IUD, with the risk of infection increasing with duration of use [5,6].

Foreign bodies in the skin reduce the bacterial inoculum required to cause infection [7]. By analogy, some researchers have concluded that the presence of an IUD in the uterus lowers host resistance to infection. If an IUD increases a woman's risk of upper genital tract infection and if her exposure to infection remains constant, then her risk of Pelvic Inflammatory Disease (PID) should remain raised throughout the duration of her IUD use. Thirty years ago, investigators showed that insertion of an IUD contaminates the endometrial cavity with bacteria [8]. Epidemiological

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studies have confirmed that the risk of upper genital tract infection associated with IUDs is temporarily linked to insertion [9].

The major complication associated with the use of medical implants such as IUDs, intravascular catheters and tubes is infection. Microbial biofilm develops when microorganisms irreversibly adhere to a surface and produce extracellular polymers that facilitate adhesion and provide a structural matrix [10]. Evidence for biofilm formation on IUDs has been demonstrated in the last 5 years [10–13]. Biofilm-associated infections caused by "sessile" microorganisms have gradually replaced the acute epidemic infections caused by "planktonic" at many clinical sites [14].

The goal of this work is to demonstrate a reproductive laboratory strain of *A. israelii*'s capacity to grow and to develop biofilms over copper surfaces in synthetic intrauterine media, resisting the local effect of the copper ions released during the oxidation process in this physiological environment.

2. Materials and methods

2.1. Bacterial revival and characterization

Freeze-dried culture of *A. israelii* from American Type Culture Collection (ATCC 10049; Manassas, VA, USA) was employed. *Actinomyces*' suspension was sub-cultured using brain-heart agar plates and they were placed inside an anaerobic jar. An incubation of 20 days at 37°C was required for *Actinomyces* growth. The colonies were identified by standard biochemical methods [15,16] and polymerase chain reaction data (not shown).

2.2. Biofilm development

A sterilized peptone enriched synthetic intrauterine media was prepared, according to the previous studies [11,17], which was then placed in a glass bioreactor adapted with an inlet for a gas mixture of N_2–CO_2 to generate anaerobic conditions. Copper plates with a surface area of 1 cm^2 were polished with abrasive paper for a 1000 SiC grade and were then immersed in test solution. The anaerobic system was placed in a water-bath to maintain a temperature of 37°C, simulating physiological conditions. N_2–CO_2 gas mixture was supplied during 24 h before inoculation of the *Actinomyces* suspension to maintain an anaerobic environment. Gas flow was kept constant during the entire experiment. To complete the system and to avoid personnel infections, a chlorine trap was made to collect the vapors coming from the reactor.

2.3. Surface analysis of A. israelii biofilm

Copper plates were exposed to the synthetic media for 172 h. After that time, specimens were analyzed for surface characterization by scanning electron microscopy (SEM) using a JEOL (JEOL, Peabody, MA, USA) JSM-6360, coupled with an Energy dispersive X-ray (EDX) analyzer (AMETEK, Mahwah, NJ, USA) used for chemical composition analysis. Specimens were fixed with 3% (v/v) glutaraldehyde for 2 h at room temperature and rinsed with phosphate buffer solution (PBS) of 0.1 M, pH=2. The specimens were then treated with 2 % (v/v) osmium tetraoxide for 1 h and rinsed again with PBS. The samples were then dehydrated with serial ethanol solutions. Samples were air dried and gold coated.

Additional specimens were analyzed by atomic force microscopy (AFM) using a Quesant Q-Scope 350 (Quesant Instruments, Agoura Hills, CA, USA) in contact mode in order to visualize the surface topography of *A. israelii* and the biofilm formed.

3. Results

3.1. A. israelii

Gram-positive strains were developed and they were confirmed under light and scanning electron microscope. Characteristic branched "spider" colonies, with sulphur granules where identical. Catalase testing was negative.

3.2. Biofilm development and characterization

Fig. 1 is a SEM micrography of biofilm formed which illustrates the Exopolysaccharide, or Exopolymeric Substances (EPS) production, bacterial distribution and sulphur granules. The spider colony distribution is confirmed. This picture also reveals the porosity of the biofilm, through which water, nutrients and copper ion flow takes place.

EDX analysis (Fig. 2) revealed the chemical composition of the biofilm, showing the presence of the copper, oxygen, carbon, potassium and sulfur. The presence of sulfur confirms that the dark pigments around the bacterial colonies were the "sulfur granules" previously reported [4]. The

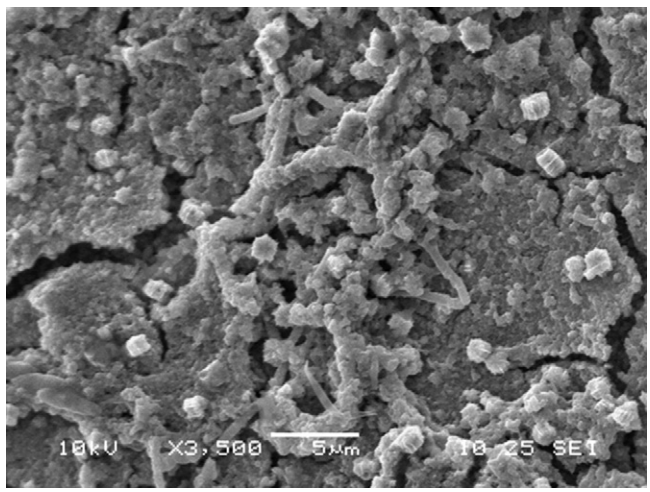

Fig. 1. SEM micrography of *Actinomyces* biofilm developed over copper plates.